COPY

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Rodney M. Richards)
Theodore Jones)
)
Serial No.: 220,108)
)
Filed: June 24, 1988)
)
For: Method and Reagents for)
Amplifying and Detecting)
Nucleic Acid Sequences)
)
Group Art Unit: 180)
)
Examiner: Scheiner)

APPELLANT'S BRIEF
(37 CRF 1.192)

93-0933

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Dear Sir:

This brief is in furtherance of the Notice of Appeal filed in this case on October 7, 1991.

The fees required under §1.17(f) and the required petition for extension of time for filing this brief and fees therefor are dealt with in the accompanying TRANSMITTAL OF APPEAL BRIEF.

This brief is transmitted in triplicate (37 CFR 1.192(a)).

This brief contains these items under the following headings and in the order set forth below (37 CRF 1.192(c)):

The final page bears the attorney's signature.

I. STATUS OF CLAIMS (37 CFR 1.192(c)(1))

A. TOTAL NUMBER OF CLAIMS IN APPLICATION

Claims in the application are: 1-39.

B. STATUS OF ALL THE CLAIMS

1. Claims cancelled: NONE.
2. Claims withdrawn from consideration but not cancelled: NONE.
3. Claims pending: 1-39.
4. Claims allowed: NONE.
5. Claims rejected: 1-39.

C CLAIMS ON APPEAL

The claims on appeal are: 1-39.

II. STATUS OF AMENDMENTS (37 CFR 1.192(c)(2))

On October 7, 1991, Applicants filed an amendment subsequent to final rejection, seeking to add claims to include the limitations that the amplification sequence being amplified be a known amplification sequence (claims 1, 14, 19, and 21) and that at least three denatured pairs of amplification probes be employed in amplification scheme of the present invention (claims 1 and 19). Applicants further sought to add new claims 40-44, depending from claims 1, 14, 19, and 21, respectively, to include the further limitation that the amplification probes be approximately the same length and contain about 2 to 30 oligonucleotides.

The Examiner refused to enter Applicants' amendment subsequent to final rejection on the basis that the amended claims "raise the issue of new matter by the recital of 'wherein said amplification probes ....contain about 2 to 30 nucleotides" (11/14/91 Office Action, page 2, lines 15-17) and further "raise new issues by

their recital of 'a known [amino acid sequence]' and 'at least three [pairs of amplification probes]'" (11/14/91 Office Action, page 3, lines 1-3), notwithstanding the fact that Applicants previously amended independent claims 14 (method) and 21 (diagnostic kit) to require a minimum of at least three pairs of amplification probes and at least two detection probes, and that the issues of probe length and amplification sequence identity had been discussed extensively in previous office actions and amendments.

III. SUMMARY OF INVENTION (37 CFR 1.192(C)(3))

A summary of the invention is provided below. All references are to the specification.

The invention relates to a two-part method for the detection of nucleic acid target sequences, wherein a detection scheme may be employed in combination with an amplification scheme to achieve the detection of low levels of target in a test sample. Amplification is accomplished by using a denatured amplification sequence of the target as a template for the contiguous hybridization of a plurality of denatured pairs of complementary amplification probes. (Page 10, line 35 to page 36, line 18. See also, Fig. 1) Each of the amplification probes is at least 2 nucleotides in length and is preferably 10 to 30 nucleotides long. (Ibid.) The probes are sufficiently complementary to the amplification sequence to enable hybridization to occur. (Page 8, lines 7-9).

The contiguously hybridized probes are ligated with a ligating reagent (page 12, line 29 to page 13, line 2) to generate an amplification product which can then be denatured from the amplification sequence (page 14, lines 6-14). The ligating reagent is preferably a ligase. (Page 13, line 32 to page 14, line 4.) Separation of the amplification product from the amplification sequence releases additional template sequence (in this case the denatured amplification product) to guide the contiguous hybridization of amplification probes in subsequent cycles of the amplification

reaction, enabling exponential accumulation of amplification product. (Page 14, lines 16-34.)

Where three or more pairs of amplification probes are employed, appropriately designed detection probes can be used to distinguish correctly assembled amplification product from incorrectly assembled spurious amplification by-product. This type of spurious by-product is formed in solution without benefit of template sequence and, therefore, is not indicative of the presence of target in the test sample. (Page 15, line 23 to page 16, line 6). If the presence of the spurious by-product is not minimized or distinguished in some way from the desired amplification product, the by-product creates serious background problems, negatively impacting sensitivity.

At least two detection probes are used in the present method, with each detection probe being complementary to a portion of each of two adjacently situated amplification probe segments of the amplification product. (Page 17, lines 15 - 25. See also, Fig. 2.) The correctly assembled amplification product serves as a template for the contiguous hybridization of the detection probes to form a detection product. (Page 18, lines 1-9.) Where *n* pairs of amplification probes are employed, it is preferred to use *n-1* detection probes. (Ibid.) The detection product can then be joined to form a ligated detection product in a manner similar to the ligation of contiguously hybridized amplification probes during amplification. (Page 18, lines 11-30.) Where the detection probes are joined, the resulting ligated detection product can, for example, be separated by polyacrylamide gel electrophoresis (PAGE). (Page 18, lines 32-36.)

Claims 1-5 are directed to the amplification procedure of the present invention. Claims 6-13 are directed to the detection procedure. The combined amplification/detection procedure is covered in claims 14-18 and 22-32. Claims 19 and 20 are directed to reagents for the amplification and detection procedures, respectively,

and claims 21 and 33-39 are directed to a kit, or set of reagents, for use in the combined amplification/detection procedure.

IV. ISSUES (37 CFR 1.192(c)(4))

1. Whether claims 3, 11, 17, and 39 are unpatentable under 35 USC §112, first paragraph, as being based on a non-enabling disclosure with respect to a thermostable ligase.

2. Whether claims 1-39 are unpatentable under 35 USC §112, first paragraph, as being based on a non-enabling disclosure with respect to other than an *E. coli* DNA ligase.

3. Whether claims 1-39 are unpatentable under 35 USC §112, first paragraph, as being based on a non-enabling disclosure with respect to the identity of an amplification sequence.

4. Whether claims 1-39 are unpatentable under 35 USC §112, first and second paragraphs, as being based on a non-enabling disclosure with respect to probe length and relative probe length.

5. Whether claims 1-39 are unpatentable under 35 USC §103 over Carr and Whiteley *et al* in view of Mullis *et al.*

V. GROUPING OF CLAIMS (37 CFR 1.192(c)(5))

The rejected claims do not stand or fall together. The following claims should be considered together with respect to the issue of obviousness: (1) claims 1-5, directed to the amplification procedure alone; (2) claims 6-13, directed to the detection procedure alone; (3) claims 14-18 and 22-32, directed to the combined amplification/detection procedure; (4) claim 19, directed to amplification reagents alone; (5) claim 20, directed to detection reagents alone; and, (6) claims 21 and 33-39, directed to a kit containing both amplification and detection reagents.

VI. ARGUMENTS (37 CFR 1.192(c)(6))

A. REJECTIONS UNDER 35 USC §112, FIRST PARAGRAPH

The Examiner rejected claims 3, 11, 17, and 39 as being unpatentable under 35 USC §112, first paragraph, for the recitation of a thermostable ligase based on Applicants' suggestion, at page 15 of the specification, that, where heat denaturation is used to separate ligated amplification product from template sequence, it is preferred to use a thermostable ligase. Specifically, the Examiner believes that claims 3, 11, 17, and 39 are not enabled, because the specification does not identify a commercial source or teach a repeatable process to obtain a thermostable ligase:

> Because heat denaturation is required in all instant examples, the thermostable enzyme is essential to the claimed invention. (04/08/91 Office Action, page 3, lines 13-15.)

The Examiner has mischaracterized the thermostable ligase as being "essential" to the present invention. In fact, the examples in the specification demonstrate the use of two different sources of the non-thermostable enzyme, *E. coli* ligase. These two different sources of enzyme are similar, but are different with respect to both purity and activity. In this regard, it is important to note that all enzymes differ in characteristics of purity and activity, both of which are reported with respect to commercially available enzymes. Applicants correctly noted, in their specification, that a thermostable ligase would be preferred for use in connection with the amplification method of the present invention, because a thermostable enzyme would eliminate the need for the addition of fresh enzyme following each heat denaturation step of the amplification procedure. In carrying out the experiments which are reported in Examples 1-8 of the present specification, Applicants employed ligases which were commercially available at the time the present application was filed. There is no basis to suggest that

Applicants' invention, as presently claimed, is not enabled simply because Applicants have noted that the commercial availability of a particular enzyme would improve performance of their method.

The Examiner also rejected claims 1-39 as being unpatentable under 35 USC §112, first paragraph, on the basis that the disclosure is enabling only for claims limited to an *E. coli* DNA ligase. According to the Examiner:

> Enablement must be commensurate with the scope of the claims. In complex or unexplored art, guidance is needed to avoid undue experimentation. Applicant's attention is directed to *In re* Colianni, 195 USPQ 150 (1972). Only the use of *E. coli* DNA ligase has been disclosed as a means of ligating hybridized amplification probes and it would have required one of ordinary skill in the art an undue amount of experimentation to have found another enzyme that would have functioned in the assay as claimed. (04/08/91 Office Action, page 3, lines 3-11.)

The Examiner's reliance on M.P.E.P. §706.03(z) in support of this rejection is misplaced, because this section of the M.P.E.P. is directed to "cases involving chemicals and chemical compounds which differ radically in their properties". In these types of situations, "it must appear in an applicant's specification either by the enumeration of a sufficient number of the members of a group or by other appropriate language, that the chemicals or chemical combinations included in the claims are capable of accomplishing the desired result." It is inappropriate to contend that the present application is enabled only for the particular type(s) of ligase demonstrated in the examples, inasmuch as the activity of a ligase may be readily predicted. As Applicants previously noted, ligases are recognized in the art as being substantially equivalent, and are sold as such. (See catalog pages from New England Biolabs, attached as Exhibit A to Amendment filed 10/07/91.) Applicants have also provided the Ampligase™ Thermostable DNA Ligase Kit specification sheet from Epicenter Technologies, demonstrating that thermostable

ligases are simply another species of the ligase genus, and are now commercially available. (See Exhibit B to Amendment filed 10/07/91.).

The Examiner's reliance on *In re* Colianni is likewise misplaced. The claims in issue in the *In re* Colianni case related to a method of mending animal bones by applying ultrasonic energy to generate heat friction in a bone fracture, thus producing a fusion-like or weld-like mend. Although the intensity of the ultrasonic energy and the length of the application were determined to be critical (there can be denaturation of the muscle or tissue in the vicinity of the fracture from the application of ultrasonic energy), the claims merely recited the application of "sufficient ultrasonic energy" to join the bone of a fracture. In contrast, the precise action of a particular ligase in Applicants' amplification/detection method does not bear the same criticality. Reaction parameters and reagent dilutions are simply adjusted to account for differences in purity and activity from one particular ligase source to another. Limiting Applicants to the particular type(s) of ligase demonstrated in the examples would be analogous to limiting the inventor in *In re* Colianni to a particular brand of ultrasonic machine.

Interestingly, the Examiner previously rejected claims 2, 3, 10, 11, 16, and 17 under 35 USC §112, first paragraph, on the basis that the disclosure is allegedly limited to a thermostable ligase:

> Claims 2, 3, 10, 11, 16, and 17 are rejected under 35 USC §112, first paragraph, as the disclosure is enabling only for claims limited to a thermostable ligase. (07/03/90 Office Action, page 4, lines 18-21, emphasis added.)

It was only after Applicants pointed out to the Examiner that the examples of the present invention in fact employ two types of *E. coli* ligases, that the Examiner reached her current position that Applicants are only entitled to claims limited to the use of an *E. coli* ligase. The Examiner appears to be confused in assuming an inherent

difference from one type of ligase to another. In fact, all ligases, by definition, achieve the same effect, namely the covalent joining (i.e., ligating) of contiguous oligonucleotide sequences. Applicants need not demonstrate the use of every possible ligase in order to obtain claims to the present invention not limited to an *E. coli* ligase.

Claims 1-39 were rejected by the Examiner under 35 USC §112, first paragraph, because the claims do not recite that the nucleotide sequence of the amplification sequence be a known nucleotide sequence. The Examiner states that:

> Claims 1-39 are not enabled for "a target nucleotide sequence" or "amplification sequence" because one would not know what synthetic oligonucleotide sequence (amplification probes) to generate and it would be critical that the probes were 100% homologous to template in order to maintain the integrity of sequence following repeated cycling. (04/08/91 Office Action, page 5, line 26 to page 6, line 3.)

Applicants have previously argued that the identity of the nucleic acid sequence need not be known in all instances. For example, it may only be necessary to know the amino acid sequence of the final protein product encoded by a particular nucleic acid sequence in order to make effective use of the present invention. In such a case, it is possible to use oligonucleotide probe mixtures, based on the protein sequence of the resulting gene product and the degeneracy of the DNA code, to identify and characterize the nucleic acid sequences responsible for encoding these gene products.

Amplification probes are defined in the specification as being complementary to amplification sequence (specification, page 7, lines 23-33), and this requirement is further set forth in the claims themselves.[1] The claims, as written, are enabled, because one must

---

[1]Note that the term "complementary" is defined in the specification as being sufficient complementarity for hybridization to occur

provide amplification probes which are complementary to the amplification sequence. In the typical diagnostic case, it would be impossible to provide these amplification probes if the amplification sequence were unknown.

Nevertheless, in order to resolve this issue, Applicants were willing to include the limitation in the claims that the nucleotide sequence of the amplification sequence be known. However, the Examiner refused to enter the proposed amendment after final, because it allegedly raises "new issues".

B. REJECTIONS UNDER 35 USC §112, FIRST AND SECOND PARAGRAPHS

The Examiner rejected claims 1-39 as being unpatentable under 35 USC §112, first and second paragraphs, based on the alleged failure of the disclosure to teach probe length and relative probe length. According to the Examiner:

> Functional language should be recited in the claims regarding the probe length and also the length of one probe relative to the others as the limitations of the specification must be read into the claims. However, the specification does not teach probe length or relative probe length and is therefor not enabling for a plurality of denatured pairs of amplification probes. As probe number and respective probe lengths are critical to the hybridization reaction as it is both individual probe length, relative probe length, and excess concentration of probe that will "drive the reaction forward". (04/08/91 Office Action, page 6, lines 10-19.)

Applicants have previously argued that, as is known to those skilled in the art, probe length is not critical with respect to the reaction kinetics of the amplification/detection system of the present

---

(specification, page 8, lines 7-9), so the Examiner's position that it is "critical that the probes [be] 100% homologous", is erroneous.

invention, but is critical only with respect to achieving specificity, in other words, distinguishing the target over the carrier DNA in a sample. For example, while both probe length and excess concentration of probe contribute to the kinetics which drive the reaction forward, it is known to those skilled in the art that an excess of probes beyond 10^8, as will typically be required to effect a ligase-based amplification system, the kinetics of the hybridization reaction becomes linear with respect to probe length. Therefore, probe length is not critical with respect to reaction kinetics in this instance.

The specification teaches that the probes should be at least two oligonucleotides long, with probe lengths of 10 and 30 nucleotides being preferred. The examples set forth in the specification employ probe lengths of 15 nucleotides. The actual probe length in a particular assay will be determined primarily on the basis of desired specificity and can readily be ascertained by one of ordinary skill in the art in light of the teachings of the present invention and teachings in the hybridization assay art which prescribe that the more complex the carrier DNA in a sample (e.g., a target with human gene carrier DNA), the longer the probe (or, in this case, the sum of the amplification probes) must be to distinguish the target over the carrier DNA. (See, for example, Helene *et al, Control of Gene Expression by Oligodeoxynucleotides Covalently Linked to Intercalating Agents and Nucleic Acid-Cleaving Reagents*, in Oligodeoxynucleotides -- Antisense Inhibitors of Gene Expression, CRC Press, Inc., 139-142 (Cohen, ed., 1989), a copy of which is attached as Exhibit C to the Amendment filed 01/03/91) wherein various means of calculating the optimum size(s) required for an oligonucleotide probe to recognize a single specific sequence in a genome are discussed.) In the case of Applicants' amplification method, it is the overall length of the contiguously hybridized amplification probes must distinguish the target sequence from, e.g., carrier DNA.

The Examiner, however, dismissed Applicants' arguments as being unconvincing because:

> Applicants' remarks regarding probe length and excess concentration ... address a single probe length or overall length of the contiguously hybridized amplification probe and not relative lengths of the three or more individual pairs of probes prior to the ligation reaction, as claimed. (04/08/91 Office Action, page 9, line 21 to page 10, line 1.)

With respect to relative probe length, Applicants argue that it is inherent from the present specification and claims that the probes need only be: (1) long enough to hybridize at the annealing temperature selected for the particular amplification scheme; and, (2) short enough to denature at the denaturation temperature selected for the particular amplification scheme. The particular relative probe lengths will be apparent to those skilled in the art, although, in most cases, it will be most convenient (but not necessary) for the probes to be approximately the same length. This is in fact taught in the examples where all amplification probes are the same length, i.e., 15 nucleotides.

Applicants are not required to state every operating parameter of the invention in the claims. This same issue was raised in *Revlon, Inc. v. Carson Products Co.*, 602 F.Supp. 1071, (S. D. N.Y. 1985), 226 USPQ 51. *aff'd* 803 F.2d 676, *cert. den.* 107 S.Ct. 671, 479 U.S. 1018, 93 L.Ed.2d 722, wherein it was stated:

> First, plaintiff asserts that the claims are ambiguous, do not state the operating parameters of the invention, and are so broad that following them would result in disintegration of the hair altogether. Although we agree that the claims are written in vague terms, we believe that the specifications elaborating upon the claims more than amply particularize the invention. The time, temperature, pH and chemical concentration limits of the invention are set forth in the specifications sufficeintly for one skilled in the art to follow. 602 F.Supp at 1098.

Importantly, Applicants set forth no fewer than 8 working examples in the present specification. These examples disclose and enable a variety of working formats of the present invention.

In addition, Applicants attempted to resolve the outstanding 35 USC §112 issues by requesting to interview the present case during the time frame of October-November, 1991. Examiner Scheiner, however, refused to grant an interview to Applicants' attorney.

C REJECTIONS UNDER 35 USC §103

Claims 1-39 were rejected by the Examiner as being unpatentable under 35 USC §103 over Carr and Whiteley *et al* in view of Mullis *et al.*

Applicants previously argued that there is no teaching or suggestion in the prior art which would lead one to combine Mullis with Carr and/or Whiteley *et al*, especially where the latter two references are directed to analytical methods which do not contemplate amplification. (Carr seeks to eliminate background from nonspecific hybridization of detection probes to carrier DNA in a target sample, while Whiteley *et al* disclose lengthening a labeled probe used for the detection of an immobilized target sequence for a similar purpose, i.e., to ameliorate the background problem presented by nonspecific and/or partial hybridization of the labeled detection probe to non-target nucleic acid.) Both the Carr and Whiteley *et al* methods take advantage of the higher T_m of the longer ligated probes to selectively denature the shorter unligated probes (which are not hybridized to target sequence), thereby removing these background-causing spuriously bound probes from the reaction mixture. In contrast, Applicants prefer to use probes having the same or similar T_m for purposes of cycling and uniformity in assay conditions.

Nevertheless, the Examiner contends that:

> ...the test of obviousness is not express suggestion of the claimed invention in any or all of the references but rather what the references taken collectively would suggest to those of ordinary skill in the art presumed to be familiar with them. (04/08/91 Office Action, page 6, line 24 to page 7, line 3, emphasis added.)

The Examiner characterizes Applicants' position as a "failure" to consider the references together, further stating:

> ...the rejection was made under 35 U.S.C 103, on the basis of what the combined teachings of the references would have suggested to one of ordinary skill in the relevant art, and not under 35 U.S.C. 102, on the basis of anticipation by any of the individual references....Again, one of ordinary skill in the art presumed to be familiar with the applied references would know that what was important was the formation of the complementary sequence and that whether one used short fragments (amplification probes) of DNA and a polymerase or short fragments of DNA and a ligase with or without a polymerase that the only thing of importance was the formation of a complementary strand which could be used in subsequent reactions as taught collectively by Mullis *et al*, Carr and Whiteley *et al.* (04/08/91 Office Action, page 7, lines 7-21, emphasis added.)

Applicants contend that they did not "fail" to collectively consider the references cited by the Examiner, but importantly, the Examiner's argument addresses only the amplification scheme standing alone, and is is not relevant to Applicants' "combined" amplification/detection system which successfully discriminates against spurious blunt-end ligated amplification by-product. There is no teaching or suggestion in the prior art which would lead one to combine Mullis with Carr and/or Whiteley *et al* to achieve the amplification/detection scheme of the present invention. The Examiner continues to rely on hindsight reconstruction in an attempt

to support her obviousness rejections of Applicants' claims. Her persistent use of the terms "would know" and "would suggest".interchangeably with the correct "would have been" standard is indicative of this approach. The correct obviousness standard is whether the invention, taken as a whole, would have been obvious to a person having ordinary skill in the art at the time the invention was made. It is Applicants position that their invention was not obvious to one of ordinary skill in the art at the time it was made.

It is important to note the impact of background caused by target-independent ligation of non-hybridized probes, because this type of "noise" can defeat the entire purpose of the amplification procedure by masking results from samples at the sensitivities which require target amplification in the first place. The occurrence of spurious by-product is particularly problematic, because the spuriously formed products serve as template in subsequent cycles to produce the exponential formation of amplification product in the absence of target sequence, thus limiting the sensitivity of the method. The background created by this type of by-product is such a serious problem in ligase-based types of amplification procedures that it can be self-defeating to the amplification method.

It is significant that others have recognized the severity of the blunt-end ligation problem in ligase-based amplification systems. European Patent Application No. 320,308 ("Backman *et al*"), for example, discloses a similar type of ligase-based amplification system, but provides only limited suggestions directed to reduction of the amount of spurious ligation product formed, namely by: (1) phosphorylating only the abutting ends of the probes; (2) using probes of unequal length; and, (3) limiting the number of amplification cycles. The first two suggestions are limited to the preferred four probe (two pair) Backman *et al* amplification system, inasmuch as only the terminal probes are available for this type of manipulation. (The Backman *et al* approach to the problem of spurious blunt-end ligated amplification by-product is not effective,

however, in Applicants' preferred amplification procedure using three or more pairs of amplification probes because the middle pair(s) of probes cannot be manipulated in the manner taught by Backman *et al.*) The last suggestion limits the sensitivity of the amplification procedure, yielding a result which is self-defeating.

It is even more significant that the blunt-end ligation problem continues to be recognized by others with respect to ligase-based amplification systems. For example, the problem was recently reported by Barringer *et al*, *Gene*, *89*, 117-122 (1990) (a copy of which is attached as Exhibit A to the Amendment filed 01/03/91), wherein it is suggested that the amount of undesired target-independent ligation can be minimized by reducing the amounts of the two reagents which participate in blunt-end ligation, namely the amplification probes and the *E. coli* ligase. Reducing the amounts of these reagents, however, negatively impacts the kinetics of the amplification reaction. Even more recently, Kwoh *et al*, *Am. Biotech. Lab.*, *8*(13) 14-25 (1990) (a copy of which is attached as Exhibit B to the Amendment filed 01/03/91), also report that the ligases used in ligase-based amplification schemes are prone to yielding blunt-end ligated by-products, because of the large excess of free nucleic acid probes required to drive the amplification reaction forward.

In contrast to the suggestions of others in the field, who continue to chase the elusive goal of reducing or minimizing the amount of blunt-end by-product formed, Applicants' method allows the blunt-end by-product to form (thereby avoiding unnecessary negative implications on the reaction efficiency) and instead discriminates the non-target-derived blunt-end ligated product from the desired target-derived amplification product. With Applicants' unique detection system, incorrectly aligned spurious blunt-end ligated amplification by-product cannot act as a template for hybridization of the detection probes. As a result, the detection product serves as an indication of only the correctly assembled amplification product, nearly all of which is traceable to the presence of target.

This unique approach enables Applicants to make use of increasing the number of pairs of probes to improve sensitivity in an assay, because the small percentage of correctly assembled by-product (which is not discriminated from the desired template-derived amplification product) decreases even further with increasing numbers of pairs of amplification probes, as set forth in the specification. In contrast, increasing the number of pairs of amplification probes is detrimental to a system which must rely on reducing or minimizing the formation of the background-causing by-product. The ability to discriminate the undesired blunt-end ligation by-product enables much greater sensitivity to be achieved by Applicants' method than is possible with the latter type of system.

Perhaps most probative of Applicants' position are the Examiner's own statements regarding the nonobviousness of the combined amplification/detection system of the present invention:

> Examiner argues that the background caused by target independent ligation of non-hybridizing probes can defeat the entire purpose of the amplification procedure by masking results from samples at the sensitivities which require target amplification in the first place and is not prevented by the instant method in any way....By increasing the number of probes (3 or more) applicants simply statistically reduce the likelihood that correctly aligned amplification product in the absence of target sequence is formed. Their method does not, however, decrease the occurrence of spurious by-product. Thus applicants' method as claimed does not appear to address the problem of background caused by incorrectly aligned spurious blunt-end ligated amplification by-product. (04/08/91 Office Action, page 8, lines 7-22, emphasis added.)

The Examiner went on to state:

> Applicants state that their detection product serves as an indication of only the correctly assembled amplification

product, nearly all of which is traceable to the presence of target.
Examiner is confused by this statement because 1) any system employing a specific probe is capable of detecting correctly assembled target or amplification product as long as respective probes sequences are specific for the aligned fragment junctions, and 2) the statement seems to imply that one can quantitatively determine how much target existed in the original sample However, the argument appears to be faulty because if the correctly aligned spurious blunt-end ligated amplification by-product (low copy number) was formed early in the cycling and subsequent exponential amplification occurred there would be just as much if not more product produced from target-independent ligated by-product than from original target of interest. (04/08/91 Office Action, page 8, line 23 to page 9, line 12, emphasis added.)

The Examiner's remarks are consistent with the teachings of the prior art which suggest that blunt ligation in a ligase-based amplification system must be addressed by reducing the amount of blunt-end ligated spurious amplification by-product formed. (See Backman *et al*, Barringer *et al*, *Gene*, *89*, 117-122 (1990), and Kwoh *et al*, *Am. Biotech. Lab.*, *8*(13) 14-25 (1990), previously cited.) In contrast, Applicants' method allows blunt-end by-product to form and instead discriminates the incorrectly assembled non-target-derived blunt-end ligated product from the desired target-derived amplification product.

However, even in the face of incontrovertible evidence, the Examiner still contends:

> The affidavit[2] has been considered but does not overcome the rejection because the use of three of more

---

[2]During a May 3, 1990 interview, Applicants provided for the Examiner's review a series of autoradiogram photographs with graphic legends which demonstrate that, where the detection system of the present invention is employed, five pairs of amplification probes provide greater sensitivity than four pairs, which, in turn,

> amplification probes does not render the invention unobvious in view of the cited art and no unexpected result or advantage has been shown when compared to said cited art. Examiner contends that although applicants repeatedly raise the "spurious blunt-end ligated amplification by-product" issue, said issue is not relevant to overcoming the rejections at hand. That is, that the "blunt-end ligation problem" has been overcome by the instant method is not pertinent to overcoming the rejection because it is obvious to do so (by employing three or more amplification probes) in view of Mullis *et al*, Carr and Whiteley *et al.* (11/14/91 Office Action, page 2, lines 5-12.)

Apparently the Examiner is now taking the position that it is obvious to use three pairs of probes, and that using three pairs of probes will overcome the blunt-end ligation problem. Three (or more) pairs of amplification probes does nothing to address the blunt-end ligation problem unless there is a way to discriminate correctly assembled product from incorrectly assembled product. This discriminatory ability is provided by Applicants in their combined amplification/detection system. Without this system, increasing the number of pairs of probes would be disastrous in a ligase based amplification system. The seemingly disastrous results expected to be encountered from increasing the total amount of blunt-end ligated amplification product points to the unobviousness of Applicants' invention.

---

provide greater sensitivity than three pairs. Applicants were informed by the Examiner that it was not necessary to produce evidence to support Applicants' position (which is set forth in the specification), because the Examiner is not allowed to question Applicants' position as stated in their specification or responses. However, in view of the Examiner contention (in the 04/08/91 Office Action) that Applicants' argument is "faulty", Applicants produced these same photographs and graphic legends in the Declaration of Rodney M. Richards, filed concurrently with the 10/07/91 Amendment.

VII. APPENDIX OF CLAIMS (37 CFR 1.192(c)(7))

The text of the claims on appeal is:

1. A method of amplifying an amplification sequence of a target nucleic acid sequence comprising:

(a) contacting said amplification sequence with an excess of a plurality of denatured pairs of amplification probes sufficient to drive the reaction forward, wherein the member probes of each of said pairs of amplification probes are complementary to each other and at least one same hybridizing member of each pair of probes is also complementary to a portion of said amplification sequence, said amplification sequence acting as a template sequence;

(b) allowing said hybridizing members of said amplification probes to hybridize to said amplification template sequence, with said amplification probes binding to said template sequence in a contiguous manner;

(c) ligating said hybridized amplification probes to form an amplification product;

(d) effecting separation of said amplification product from said template sequence; and,

(e) repeating steps (a) through (d), wherein said amplification product also acts as a template sequence in subsequent cycles of steps (a) through (d).

2. The method of claim 1 wherein said hybridized amplification probes are joined together by the action of a ligase.

3. The method of claim 2 wherein said ligase is a thermostable ligase.

4. The method of claim 1 wherein said hybridized amplification probes are joined together through a chemical reaction.

5. the method of claim 1 wherein at least three pairs of amplification probes are used.

6. A method for detecting an amplification product, having three or more ligated amplification probe segments, comprising:

(a) contacting said amplification product with at least two detection probes, wherein at least one of said detection probes is labeled, and wherein each of said detection probes is complementary to a portion of each of two of said ligated amplification probe segments which are adjacently situated in said amplification product;

(b) allowing each of said detection probes to hybridize to two adjacently situated amplification probe segments of said amplification product, with said detection probes binding to said amplification product in a contiguous manner to form a detection product;

(c) detecting the presence of said detection product through the presence of said label.

7. The method of claim 6 further comprising separating said unhybridized labeled detection probes from said hybridized detection product.

8. The method of claim 7 wherein one of said detection probes is labeled with a first proximity label and the other of said detection probes is labeled with a second proximity label.

9. The method of claim 6 wherein said hybridized detection probes are ligated to form a ligated detection product.

10. The method of claim 9 wherein said hybridized detection probes are joined together by the action of a ligase.

11. The method of claim 10 wherein said ligase is a thermostable ligase.

12. The method of claim 9 wherein said hybridized detection probes are joined together through a chemical reaction.

13. The method of claim 9 wherein one of said detection probes is labeled with a detectable label and the other of said detection probes is labeled with a means for removing said ligated detection product from solution.

14. A method for detecting an amplification sequence of a target nucleic acid sequence which may be present in a test sample comprising:

(a) contacting said test sample with an excess of at least three denatured pairs of nucleic acid amplification probes sufficient to drive the reaction forward, wherein the member probes of each of said pairs of amplification probes are complementary to each other and at least one same hybridizing member of each pair of probes is also complementary to a portion of said amplification sequence, said amplification sequence acting as a template sequence;

(b) allowing said hybridizing members of said amplification probes to hybridize to said amplification template sequence, with said amplification probes binding to said template sequence in a contiguous manner;

(c) ligating said hybridized amplification probes to form an amplification product;

(d) effecting separation of said amplification product from said template sequence;

(e) repeating steps (a) through (d), wherein said amplification sequence also acts as a template sequence in subsequent cycles of steps (a) through (d);

(f) contacting said amplification product with at least two detection probes, wherein at least one of said detection probes is labeled, and wherein each of said detection probes is complementary to a portion of each of two of said amplification probe segments which are adjacently situated in said amplification product;

(g) allowing each of said detection probes to hybridize to two adjacently situated amplification probe segments of said amplification product, with said detection probes binding to said amplification product in a contiguous manner to form a detection product;

(h) detecting the presence of said hybridized detection product through the presence of said label.

15. The method of claim 14 further comprising:

(a) causing said hybridized detection probes to join together to form a ligated amplification product; and,

(b) detecting the presence of said ligated detection product.

16. The method of claim 14 wherein said hybridized amplification probes are joined together by the action of a ligase.

17. The method of claim 15 wherein said ligase is a thermostable ligase.

18. The method of claim 14 wherein said hybridized amplification probes are joined together through a chemical reaction.

19. A reagent for use in the amplification of an amplification sequence comprising an excess of a plurality of pairs of nucleic acid amplification probes sufficient to drive the reaction forward, wherein the member probes of each pair of amplification probes are complementary to each other and at least one same hybridizing member of each pair of amplification probes is also complementary to a given portion of said amplification sequence, with the nucleic acid sequence of each pair of amplification probes selected to be complementary to said amplification sequence, said amplification sequence acting as a template sequence, the amplification probes being capable of hybridizing to the template sequence in a contiguous manner sufficiently adjacent to each other to enable the probes to be ligated to form a detection product.

20. A reagent for use in the detection of an amplification product, wherein said amplification product has three or more ligated amplification probe segments, said reagent comprising at least two nucleic acid detection probes, wherein each of said detection probes is complementary to a portion of each of two of said

ligated amplification probe segments which are adjacently situated in said amplification product, with at least one of said detection probes being provided with a label, the detection probes being capable of hybridizing to said amplification product in a contiguous manner to form a detection product.

21. A kit for use in the detection of an amplification sequence of a target nucleic acid sequence which may be present in a test sample comprising:

(a) an excess of at least three pairs of amplification probes sufficient to drive the reaction forward, wherein the member probes of each of said pairs of amplification probes are complementary to each other and at least one same hybridizing member of each pair of amplification probes is also complementary to a portion of an amplification sequence of said target nucleic acid sequence, said amplification sequence acting as a template sequence, and said amplification probes being capable of hybridizing to said template sequence in a contiguous manner having a gap of no more than one nucleotide between said amplification probes, such that said amplification product is made up of ligated amplification probe segments; and,

(b) at least two detection probes, wherein at least one of said detection probes is labeled, and wherein each of said detection probes is complementary to a portion of each of two of said amplification probe segments of said amplification product which are adjacently situated in said amplification product, with at least one of said detection probes being provided with a label, said detection probes being capable of hybridizing to said amplification product in a contiguous manner to form a detection product.

22. The method of claim 14 wherein said amplification sequence is contacted with n pairs of amplification probes and said amplification product in contacted with n-1 detection probes.

23. The method of claim 22 wherein said amplification sequence is contacted with 3 pairs of amplification probes and said amplification product in contacted with 2 detection probes.

24. The method of claim 22 wherein said amplification sequence is contacted with 4 pairs of amplification probes and said amplification product in contacted with 3 detection probes.

25. The method of claim 22 wherein said amplification sequence is contacted with 5 pairs of amplification probes and said amplification product in contacted with 4 detection probes.

26. The method of claim 16 wherein said amplification sequence is contacted with n pairs of amplification probes and said amplification product in contacted with n-1 detection probes.

27. The method of claim 26 wherein said amplification sequence is contacted with 3 pairs of amplification probes and said amplification product in contacted with 2 detection probes.

28. The method of claim 26 wherein said amplification sequence is contacted with 4 pairs of amplification probes and said amplification product in contacted with 3 detection probes.

29. The method of claim 26 wherein said amplification sequence is contacted with 5 pairs of amplification probes and said amplification product in contacted with 4 detection probes.

30. The method of claim 17 wherein said amplification sequence is contacted with n pairs of amplification probes and said amplification product in contacted with n-1 detection probes.

31. The method of claim 30 wherein said amplification sequence is contacted with 3 pairs of amplification probes and said amplification product in contacted with 2 detection probes.

32. The method of claim 30 wherein said amplification sequence is contacted with 4 pairs of amplification probes and said amplification product in contacted with 3 detection probes.

33. The method of claim 30 wherein said amplification sequence is contacted with 5 pairs of amplification probes and said amplification product in contacted with 4 detection probes.

34. The kit of claim 21 wherein n pairs of amplification probes are provided in combination with n-1 detection probes.

35. The kit of claim 34 wherein 3 pairs of amplification probes are provided in combination with 2 detection probes.

36. The kit of claim 35 wherein 4 pairs of amplification probes are provided in combination with 3 detection probes.

37. The kit of claim 35 wherein 5 pairs of amplification probes are provided in combination with 4 detection probes.

38. The kit of claim 37 further comprising a ligase.

39. The kit of claim 38 wherein said ligase is a thermostable ligase.

Date: April 7, 1992

Julia E. Abers

Julia E. Abers
Reg. No. 31,222
Phone: (805) 499-5725, Ext. 3162

Please send correspondence pertaining to this application to:

Steven M. Odre/JEA
Amgen Inc.
1840 Dehavilland Drive
Thousand Oaks, CA 91320-1789

#23 in B. 04/30/92

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Rodney M. Richards & Theodore Jones

Serial No.: 07/ 220,108 Group No.: ~~187~~ 182

Filed: 6-24-88 Examiner: Scheiner, L.

For: Mehtod and Reagents for Amplifying and Detecting Nucleic Acid Sequences

Commissioner of Patents and Trademarks
Washington, D.C. 20231

18m
12/4/91

TRANSMITTAL OF APPEAL BRIEF (PATENT APPLICATION—37 CFR 192)

1. Transmitted herewith in triplicate is the APPEAL BRIEF in this application with respect to the Notice of Appeal filed on 10-7-91.

NOTE: "The appellant shall, within 2 months from the date of the notice of appeal under § 1.191 in an application, reissue application, or patent under reexamination, or within the time allowed for response to the action appealed from, if such time is later, file a brief in triplicate." 37 CFR 1.192 [emphasis added].

2. **STATUS OF APPLICANT**

This application is on behalf of

- [X] other than a small entity
- [ ] small entity

verified statement:

- [ ] attached
- [ ] already filed

3. **FEE FOR FILING APPEAL BRIEF**

Pursuant to 37 CFR 1.17(f) the fee for filing the Appeal Brief is:

[ ] small entity	$ 120.00
[X] other than a small entity	$ 240.00

Appeal Brief fee due $ 240.00

CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper (along with any referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Beverly Arties
(Type or print name of person mailing paper)

Date: 4-7-92

Beverly Arties
(Signature of person mailing paper)

1,150.00CH

DF11376 04/15/92 07220108

4. EXTENSION OF TERM

NOTE: The time periods set forth in 37 CFR 1.192(a) are subject to the provision of § 1.136 for patent applications 37 CFR 1.191(d). Also see Notice of November 5, 1985 (1060 O.G. 27).

The proceedings herein are for a patent application and the provisions of 37 CFR 1.136 apply.

(complete (a) or (b) as applicable)

(a) [X] Applicant petitions for an extension of time under 37 CFR 1.136 (fees: 37 CFR 1.17(a)-(d)) for the total number of months checked below:

	Extension (months)	Fee for other than small entity	Fee for small entity
[ ]	one month	$100.00	$50.00
[ ]	two months	$300.00	$150.00
[ ]	three months	$730.00	$365.00
[x]	four months	$1,150.00	$575.00

Fee $ 1,150.00

If an additional extension of time is required please consider this a petition therefor.

(check and complete the next item, if applicable)

[ ] An extension for _____ months has already been secured and the fee paid therefor of $_____ is deducted from the total fee due for the total months of extension now requested.

Extension fee due with this request $1,150.00

or

(b) [ ] Applicant believes that no extension of term is required. However, this conditional petition is being made to provide for the possibility that applicant has inadvertently overlooked the need for a petition and fee for extension of time.

4. TOTAL FEE DUE

The total fee due is:

Appeal brief fee $ 240.00

Extension fee (if any) $ 1,150.00

TOTAL FEE DUE $ 1,390.00

5. FEE PAYMENT

[ ] Attached is a check in the sum of $_____

[x] Charge Account No. 01-0519 the sum of $ 1,390.00

A duplicate of this transmittal is attached.

6. FEE DEFICIENCY

NOTE: If there is a fee deficiency and there is no authorization to charge an account, additional fees are necessary to cover the additional time consumed in making up the original deficiency. If the maximum, six-month period has expired before the deficiency is noted and corrected, the application is held abandoned. In those instances where authorization to charge is included, processing delays are encountered in returning the papers to the PTO Finance Branch in order to apply these charges prior to action on the cases. Authorization to change the deposit account for any fee deficiency should be checked. See the **Notice of April 7, 1986,** *1065 O.G. 31-33.*

☒ If any additional extension and/or fee is required, this is a request therefor and to charge Account No. 01-0519.

AND/OR

☒ If any additional fee for claims is required, charge Account No. 01-0519.

Julia E Abers

SIGNATURE OF ATTORNEY

Julia E. Abers

Type or print name of attorney

Reg. No.: 31,222

Tel. No.: (805) 499-5725 Ext. 3162

1840 Dehavilland Drive

P.O. Address

Thousand Oaks, CA 91320